

Levels of tryptase, chymase, and FcεRIα messenger RNA in human skin are unchanged after IgE-dependent stimulation of cutaneous mast cells in vivo

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IgE-dependent activation of mast cells is associated with secretion of tryptase and chymase, along with other granule mediators, and internalization of the high-affinity receptor for IgE, FcεRI. We hypothesized that messenger RNA levels in mast cells corresponding to these molecules might be dramatically elevated within 24 hours of mast cell activation in preparation for replacement of lost proteins.

METHODS

Ultrastructural examinations of human lung-derived mast cells within 24 hours of activation show increased smooth and rough endoplasmic reticulum,¹ providing support for our hypothesis. Consequently, 3 mm punch biopsy specimens were taken from anesthetized (with 2% lidocaine) skin sites 6 hours and 24 hours after intradermal injection (0.03 ml) of either an antigen (grass or ragweed extract, 100 PNU/ml; Greer Laboratories, Lenoir, N.C.) to which the subject was sensitive or a diluent control as previously described.² Each biopsy specimen was immediately snap-frozen in liquid nitrogen. Total RNA was extracted by use of RNeasy B (Tel-Test, Inc., Friendswood, Texas), a modified guanidinium thiocyanate technique,³ as described by the manufacturer. Each sample of target RNA was subjected to competitive reverse-transcription polymerase chain reaction (RT-PCR) with gene-specific primers and competitor molecules consisting of complementary RNA for tryptase, chymase, the α subunit of FcεRI, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH), as described previously for tissue-derived mast cells and peripheral blood basophils.⁴ The cRNA competitor molecules were generated from corresponding complementary DNAs that had small portions of the sequence deleted from a region internal to the primers. The use of cRNA competitor molecules at the RT step corrects for variability in the efficiency of RT and of PCR in different experiments. A ratio (R) of target to competitor PCR product

Abbreviations used

cRNA:	Complementary RNA
FcεRI:	High-affinity receptor for IgE
G3PDH:	Glyceraldehyde-3-phosphate dehydrogenase
mRNA:	Messenger RNA
R:	Ratio of target to competitor PCR product
RT-PCR:	Reverse transcription-polymerase chain reaction

was calculated, and this represents an index of the relative amounts of mRNA. The molar concentration of cRNA equals the concentration of mRNA in the RT-PCR reaction mixture when the R value is equivalent to a molar target-to-competitor ratio of 1. To determine target mRNA concentrations when a limiting amount of cellular RNA is available, R_n values were first determined at a single competitor cRNA concentration (C_n). A C₁ value was next calculated for the R₁ value corresponding to a target-to-competitor molar ratio of 1. R₁ values were 1.32 for tryptase, 1.58 for chymase, 1.89 for FcεRIα, and 1.35 for G3PDH. C₁ was calculated according to the following formula:

$$C_1 = C_n \times \left(\frac{R_1}{R_n} \right)^{\frac{1}{K}}$$

where K is the slope constant corresponding to a particular molecule and to a set of reaction conditions as calculated from the following formula:

$$\log(R) = K \times \log(C) + B$$

As described previously,⁴ log(R) shows a linear relationship to log(C) with a slope K and a y-axis intercept B. K values (mean ± SD) determined in separate experiments⁴ with mRNA from HMC-1 cells, KU812 cells, peripheral blood basophils, and tissue mast cells dispersed from lung and skin were as follows: tryptase, -1.24 ± 0.17, n = 12; chymase, -1.21 ± 0.11, n = 6; FcεRIα, -1.05 ± 0.12, n = 6; and G3PDH, -1.24 ± 0.25, n = 19. Values for tryptase, chymase, and FcεRIα were then normalized to the corresponding value for G3PDH to obtain the relative amount of each mRNA molecule in the skin biopsy specimen. This latter step diminishes the variability arising from differences in the cellular RNA content of different biopsy specimens.

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Received for publication May 7, 1996; revised June 24, 1996; accepted for publication June 25, 1996.

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J Allergy Clin Immunol 1997;99:224-6.

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0091-6749/97 \$5.00 + 0 1/176091

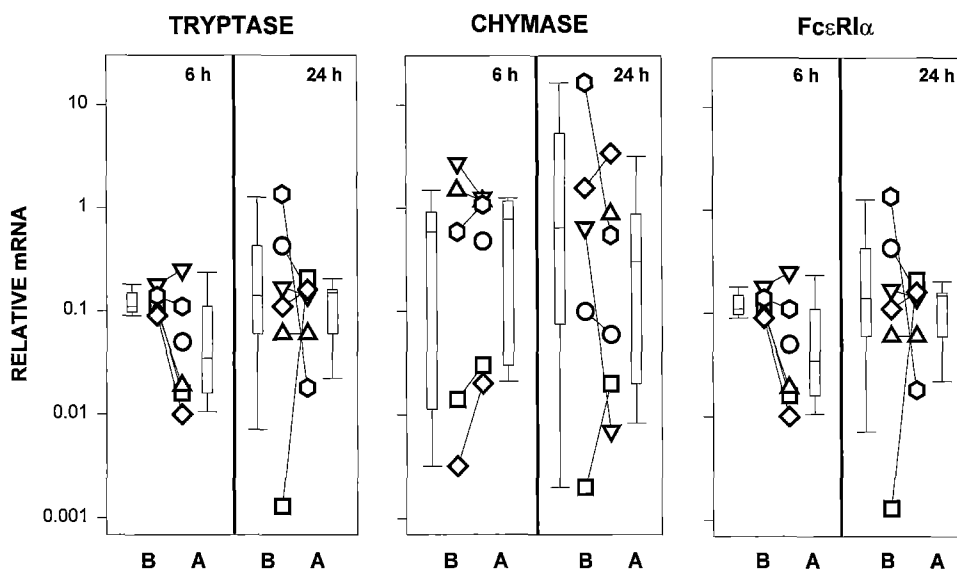


FIG. 1. Relative mRNA content of tryptase, chymase, and the α subunit of the Fc ϵ RI receptor in buffer-challenged (B) and allergen-challenged (A) human skin. For each RT-PCR reaction, 500 ng of RNA was used. R_t values were calculated for tryptase, chymase, Fc ϵ RI α , and G3PDH; mRNA values for mast cell-related markers were normalized to those of G3PDH to obtain relative mRNA values, as described in the text. Each subject is shown with a distinct symbol. Box and whisker plots show the median and the 25th and 75th percentiles; error bars show the 5th and 95th percentiles.

RESULTS

Each of six atopic subjects (four men) with quiescent respiratory allergic diseases and no skin disease were challenged by intradermal injection of allergen (100 PNU/ml) at two sites and of diluent at two sites. Wheal (12 to 25 mm) and flare (31 to 50 mm) responses occurred at each allergen site 20 minutes after challenge with antigen, whereas buffer-challenged sites at this time point showed 0 to 3 mm wheals and no flares. Induration (>10 mm diameter) was observed in all allergen-challenged sites 6 hours later and in three of the six allergen-challenged sites at 24 hours. No buffer-challenged site exhibited detectable induration (≥ 1 mm) at either time point. After RNA extraction, relative mRNA levels of chymase, tryptase, and Fc ϵ RI α were determined as shown in Fig. 1. No mRNA was detected in the 6-hour buffer control specimen in one subject, which suggested that RNA had been degraded. This data point was not considered in the analyses. Also, no RT-PCR product for target or competitor chymase nucleic acid was found in one 24-hour buffer-challenged site, and this data point was also deleted from the analyses. Because of limiting quantities of RNA, each assay could be performed only once. Although there was a tendency for Fc ϵ RI α mRNA levels to increase at the 24-hour antigen-challenge site compared with the buffer-challenge site and for the tryptase mRNA levels to decrease at the 6-hour antigen-challenge site compared with the buffer-challenge site, no statistically significant differences in the median values between antigen-challenge and buffer-challenge sites were found ($p > 0.2$; Wilcoxon

signed-rank test for nonparametric data). Also, there was no significant correlation between levels of mRNA for the granule mediators (tryptase and chymase) and mRNA of the membrane receptor (Fc ϵ RI α) in the biopsy specimens.

DISCUSSION

In this study we found highly variable amounts of mRNA for tryptase, chymase, and Fc ϵ RI α in the skin of different subjects. This variability was also found in a previous study of mast cells dispersed from skin.⁴ In part, this may reflect variations in the relative mast cell content in tissue samples taken from different sites, as suggested by the variations in mRNA content of the 6-hour and 24-hour buffer-challenge sites (assumed to be unstimulated) in the same individual. Also, as found previously in studies with dispersed mast cell preparations in which data could be normalized to the number of mast cells, mRNA content per mast cell varies from individual to individual. Finally, the possibility that basophils were recruited into the allergen-challenge sites by 24 hours and influenced mRNA levels should be considered. Although Fc ϵ RI α mRNA levels in mast cells and basophils are comparable, tryptase mRNA levels are 10^5 times lower in basophils than in mast cells, and chymase mRNA is undetectable in basophils.⁴ Fc ϵ RI α mRNA levels were higher in five of six allergen-challenged sites than in corresponding buffer-challenged sites at 24 hours. Previous studies of allergen challenge in which the skin-chamber technique was used suggested

that basophils may be recruited into these sites as early as 1 hour after challenge.⁵ On the other hand, the FcεRIα R1 values at the 24-hour antigen-challenge sites were no higher than those at the 6-hour allergen- and buffer-challenge sites. Thus it is unlikely that basophils substantially affected the cellular levels of FcεRIα mRNA under the conditions of this study.

We conclude from these data that mRNA levels for tryptase, chymase, and FcεRIα do not change substantially in mast cells in vivo within 24 hours of allergen-mediated activation. This suggests that increases in mRNA levels for these molecules are not likely required to replenish depleted stores of these proteins in mast cells recovering from degranulation. Instead, translation and posttranslational processing may be more important. One consideration in this analysis is the percentage of mast cells actually activated in vivo by the allergen challenge. For example, if only a small percentage of mast cells degranulated (a situation we consider unlikely), a consistent increase in mRNA levels among these cells might be masked by the mRNA levels in the larger numbers of unstimulated mast cells. Another consideration is mRNA turnover. mRNA content at any one time reflects the result of both synthesis and degradation. Therefore our findings do not exclude the possibility

that synthesis of new mRNA for these proteins increases after mast cell activation but do suggest that the quantity of new mRNA does not substantially raise the concentration of that particular mRNA, perhaps because mRNA degradation increases proportionately.

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